Gamma Ray Processing to Destroy *Staphylococcus aureus* in Mechanically Deboned Chicken Meat

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ABSTRACT

Gamma radiation doses of 0.26 kGy and 0.36 kGy, administered *in vacuo* at 0°C, destroyed 90% of log-phase and stationary-phase colony forming units (CFU) of *Staphylococcus aureus* ATCC 13565 (FDA 196E), respectively, in mechanically deboned chicken meat (MDCM). Samples inoculated with $10^{3.9}$ CFU/g of *S. aureus* were treated with gamma radiation *in vacuo* at 0°C and then held for 20 hr at 35°C (abusive storage). Viable CFU were found in samples irradiated to 0.75 kGy but not in those irradiated to 1.50 kGy either before or after storage. Enterotoxin was not detected in irradiated MDCM. A predictive equation was developed for the response of *S. aureus* in MDCM to radiation dose and irradiation temperature.

Key Words: chicken, MDCM, gamma ray, irradiation, staphylococcus, deboning

INTRODUCTION

STAPHYLOCOCCUS AEREUS is an important source of food poisoning throughout the world. Between 1983 and 1987 *S. aureus* was responsible for 47 outbreaks and 3,181 cases of foodborne disease in the U.S. (Bean et al., 1990), representing 7.4% of the total number of bacterial foodborne disease cases during that time. Meats (beef, ham, pork, turkey, poultry salad) were identified as the etiological agents in 42.2% of the outbreaks. *S. aureus* was reported as a contaminant of poultry carcasses (Adams and Mead, 1983; Mead et al., 1989; Notermans et al., 1982; Harvey et al., 1982) and of mechanically deboned poultry meat (Kumar et al., 1986; Lillard et al., 1984; Fuchs et al., 1980). The use of ionizing radiation treatments of poultry meat for the purpose of eliminating foodborne pathogens was approved in 1990 (Anon., 1990). Such treatments would include those for eliminating staphylococci.

Published information on the effects of gamma radiation on staphylococci, particularly in meat, is very limited. Erdman et al. (1961) studied the radiation resistance of 6 strains of 24 hr cultures of *S. aureus* to gamma radiation when suspended in broth and of one strain suspended in chopped beef. Samples were irradiated at "room" temperature using a cobalt^{60} source with a dose rate of 0.05 kGy/min. They did not report D-values; however, a D-value could be extrapolated from the published regression curve for the destruction of *S. aureus* in broth, which we estimated to be 0.52 kGy. Erdman et al. (1961) reported that *Staphylococcus* was more radiation resistant than salmonellae, and that cells incorporated into chopped beef were significantly more resistant than those suspended in broth. Tiwari and Maxcy (1972) reported a D-value for stationary phase cells of *S. aureus* of 0.43 kGy when irradiated in nutrient broth. However, those results are difficult to interpret because neither the temperature nor the atmosphere during irradiation was indicated.

The objectives of our study were to determine in MDCM; (1) the effects of radiation dose, and the temperature and atmosphere during irradiation on the survival of a strain typically associated with food poisoning, *S. aureus* ATCC 13565 (FDA 196E); (2) to compare the radiation resistance of a mixture of suspended strains of *S. aureus* to that of *S. aureus* ATCC 13565; (3) to develop equations that would predict the effects of radiation on *Staphylococcus* survival at various gamma radiation doses and temperatures; (4) to determine the effect of the growth phase of *S. aureus* ATCC 13565 on its gamma radiation D-value and (5) to challenge with temperature abuse the effectiveness of gamma radiation doses below 3.0 kGy for the elimination of *Staphylococcus* and prevention of enterotoxin formation.

MATERIALS & METHODS

Culture

S. aureus ATCC 13565 (FDA 196E), from the American Type Culture Collection, Rockville, MD, was used and was cloned and transferred monthly on Tryptic Soy Agar (Difco, Detroit, MI). This strain originated as a clinical isolate from a case of staphylococcal food poisoning associated with ham. Four other strains of *S. aureus* designated B121, B124, B767, and 196E were obtained from the USDA, Eastern Regional Research Center culture collection. The cultures were maintained and cloned on TSA with incubation at 35°C. The cultures were propagated for 4 hr for mid-log phase cells or 16 to 18 hr for stationary-phase cells, as appropriate, in 100 mL of Tryptic Soy Broth (TSB) (Difco, Detroit, MI) contained in a 500 mL baffled DeLong style culture flask, which was agitated at 150 rpm and incubated at 35°C. A 1 mL aliquot of a stationary-phase culture in TSB was used to inoculate 100 mL of TSB for propagation. Culture purity and identity were verified with Gram stains, hemagglutination tests (BBL® Staphyloslide™, Becton Dickinson and Co., Cockeysville, MD), and by GPI identification card results (Vitek Systems, Hazelwood, MO).

Substrate

Samples were obtained on the day MDCM was delivered to a major manufacturer of chicken frankfurters. They were mixed well and vacuum packaged in 100 g units in sterile Number 400 polyethylene Stomacher® bags (Tekmar Co., Cincinnati, OH), which were then vacuum packaged within Freshstuff® bags (American National Can Company, Des Moines, Iowa). (The bags have an oxygen permeability of 0.6–0.8 cc/645.2 cm^2/24 hr at 22.8°C, 50% R.H. and a water transmission rate of 0.3–0.4 g/645 cm^2/24 hr at 37.8°C, 90% R.H.). The chicken meat was then sterilized with a gamma radiation dose of 42 kGy at −50°C and stored frozen at −20°C. Prior research (Thayer et al., 1987) had demonstrated that such treatments did not significantly alter the wholesomeness or nutritional characteristics of chicken meat. The average proximate analysis for this meat was 66.1% moisture, 5.9% fat, 10.7% protein, 1.1% ash, and 16.2% carbohydrate. The proximate analyses were performed in duplicate by a USDA certified commercial laboratory.

Irradiation

The gamma radiation source was ^{137}Cs with a source strength of 134,000 Ci, producing a dose rate of 0.12 kGy/min. The dosimetry and dose distribution for this radiation source were described by Shieh et al. (1985). Routine dosimetry was conducted with radiochromic dosimeters (Far West Technology Inc., Goleta, CA), which were referenced to National Physical Laboratory standard dosimeters (Middlesex, United Kingdom). Variations in absorbed dose were minimized by placing very thin (less than 2 mm) samples within a uniform portion of the radiation field. The total mass of the samples being treated at

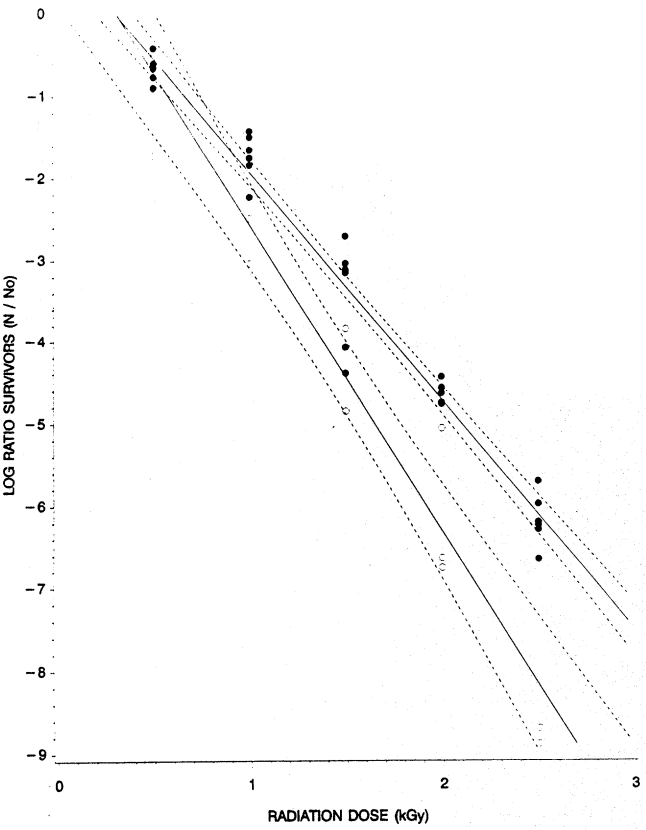

Fig. 1—Response of stationary phase cfu (•) or mid-log phase CFU (○) of S. aureus *ATCC 13565 (FDA 196E) to gamma radiation in mechanically deboned chicken meat. The 95% confidence intervals for each linear regression are indicated by the dotted lines.*

any one time was small, (usually not exceeding 20g). Samples were maintained ±0.5°C by the injection of liquid nitrogen into the irradiation chamber.

Gamma radiation D-values

Sterile MDCM was mixed well with $10^{9.5}$ CFU/g of stationary-phase *S. aureus* ATCC 13565 suspended in buffered 0.10% peptone (Difco, Detroit, MI). Samples of 5.0±0.05g of the inoculated MDCM were aseptically transferred to sterile Number 400 polyethylene Stomacher® bags. The inoculated meat was spread uniformly over an area of about 10 × 10 cm within the bag and heat-sealed either *in vacuo* (−0.95 bar) or with air in the bag. Each Stomacher bag containing a sample was vacuum packaged within a Freshstuff® bag to prevent oxygen absorption by vacuum packed samples and to provide additional microbiological security for all samples during the treatment with radiation. Samples were exposed to gamma ray doses at 0°C in increments of 0.50 kGy to a maximum of 3.0 kGy. Mid-log-phase *S. aureus* ATCC 13565 cells in vacuum packaged MDCM at 0°C were used in a second study. Those studies were replicated at 3 different times.

Each of the 4 *S. aureus* strains from the local culture collection (B121, B124, 196E, and B767) was cultured in TSB and harvested at the stationary-phase, as described above. The cells from each culture were centrifuged and resuspended in 0.1% buffered peptone, mixed with cells from the other 3 strains, centrifuged, and resuspended in 0.1% buffered peptone. D-values were determined for the pooled strains, as described above, in buffered peptone (at $10^{9.7}$ CFU/mL) and when mixed with MDCM (at $10^{9.5}$ CFU/g). This study was replicated at two different times.

Effect of radiation dose and temperature of irradiation

Sterile MDCM was mixed well with $10^{9.3}$ CFU/g of stationary-phase *S. aureus* ATCC 13565 and 20 replicate samples vacuum packaged in 5.0±0.05g amounts as described. The central composite response-surface design included 2 replicate treatments at 0.0 kGy at irradiation temperatures of −20, 0, and +20°C. There were 5 replicate treatments at 1.5 kGy at an irradiation temperature of 0°C. Single samples were used at −20°C at radiation doses of 1.5 and 3.0 kGy, at −10°C and 0.75 and 2.25 kGy, at 0°C and 3.0 kGy, at +10°C and 0.75 and 2.25 kGy, and at +20°C and 1.5 and 3.0 kGy. Regression techniques were used to fit a second order response-surface model to the data allowing prediction of *S. aureus* ATCC 13565 survival following a given treatment.

Challenge study

Sterile MCDM was inoculated with $10^{3.9}$ washed cfu per gram of *S. aureus* ATCC 13565 and vacuum packaged in 5.0 g amounts. Samples were irradiated to 0, 0.75, 1.50, 2.25, and 3.0 kGy at 0°C. One sample was analyzed immediately after irradiation; the second was incubated at 35°C for 20 hr before analysis for the cfu of *S. aureus* ATCC 13565 and for the presence of staphylococcal enterotoxins A, B, C_1, C_2, C_3, D, and E by an enzyme-linked immunosorbent assay procedure (Report™, Microbiology Products, 3M Health Care, St. Paul, MN). The study was replicated at two different times.

Microbiological assay

Samples diluted in sterile 0.1% buffered peptone were blended in a Stomacher, and serial dilutions were prepared in buffered 0.1% peptone. Standard pour-plates were prepared using TSA and incubated for 24 hr at 35°C. The CFU on 3 Petri plates at a dilution giving 30 to 300 colonies were counted using a Biotran II® automated colony counter (New Brunswick Scientific Co., Inc., Edison, NJ).

Statistical analysis

Cultural responses were expressed as the logarithm of the cfu/g. For each experiment, the average (N) cfu values for the 3 plate counts obtained for each replicate sample was determined and divided by the average of the 3 zero-dose values (N_o) to give a survivor value (N/N_o). The $\log_{10}$ survivor values ($\log_{10}(N/N_o)$) were then used for subsequent calculations. The D-values (dose in kGy resulting in a 90% reduction of viable cfu) were the reciprocals of the slopes of the linear regressions of the log survivor values determined by least squares analyses. The zero-dose values were excluded from the calculation of the regression to exclude shoulder effects as described by Thayer et al. (1990). Regression techniques were used to fit second-order reponse-surface models (Draper and Smith, 1981). Statistical calculations were performed with the general linear models procedure of the SAS statistical package (Freund et al., 1986; SAS Institute, 1987). Regressions were tested for differences by analysis of covariance.

RESULTS

Gamma radiation D-values

The gamma radiation D-values for stationary-phase *S. aureus* ATCC 13565 in MDCM were 0.37±0.02 kGy in the presence of air and 0.35±0.01 kGy *in vacuo*. The values were not significantly different. When the results from studies in both the presence and absence of air were combined, the D-value for stationary phase *S. aureus* ATCC 13565 in MDCM was 0.36±0.01 kGy. The gamma radiation D-value for mid-log phase *S. aureus* ATCC 13565 in vacuum packed MDCM was 0.27±0.02 kGy and was significantly different ($P<0.0001$) from that for stationary phase CFU (Fig. 1).

The gamma radiation D-values for the mixture of the *S. aureus* strains 196E, B121, B124 and B767 were 0.10±0.004 kGy when suspended in peptone and 0.47±0.01 kGy when mixed with MDCM. The regressions from which these D-values were calculated were significantly different ($P<0.0001$) (Fig. 2).

Effect of radiation dose and temperature of irradiation

The means of the estimates of the CFU of *S. aureus* ATCC 13565 expressed as their logarithm_{10}, from the central composite response-surface study of the effects of gamma radiation on *S. aureus* in MDCM were determined (Table 1). Statistical

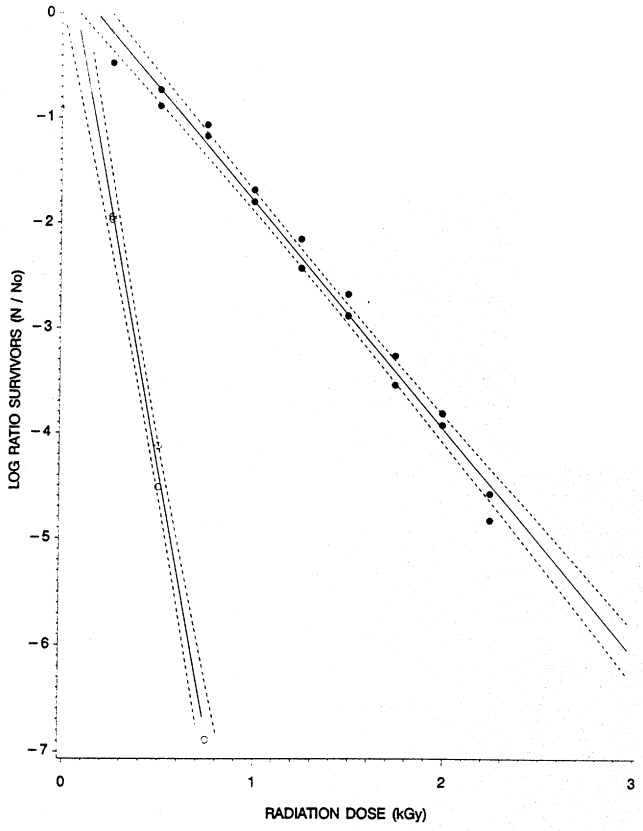

Fig. 2—Response of equal mixtures of the stationary phase CFU of S. aureus *strains B121, B124, 196E and B767 to gamma radiation when suspended in mechanically deboned chicken meat (●) or in 0.1% peptone water (○). The 95% confidence intervals for each linear regression are indicated by the dotted lines.*

Table 1—Logarithm of average colony forming units of S. aureus *ATCC 13565 in mechanically deboned chicken meat surviving treatment with gamma radiation*

Irradiation temp °C	Gamma radiation dose (kGy)				
	0	0.75	1.50	2.25	3.00
	Log_{10} CFU				
−20	9.30		7.38		3.15
−10		8.13		4.97	
0	9.23		5.49		0
+10		4.97		3.96	
+20	9.25		5.81		0.86

analysis revealed significant effects for radiation dose ($P < 0.0001$), temperature of irradiation ($P < 0.012$), and for the interaction of radiation dose with temperature of irradiation ($P < 0.016$). Significant quadratic effects were also revealed for (radiation dose)^2 ($P < 0.004$) and (temperature of irradiation)^2 ($P < 0.014$). The corresponding response surface is presented in Fig. 3. This response surface is described by the following equation:

$$\text{Log Survivors } (N/N_o) = 0.500 - 1.328\,(\text{kGy}) - 0.002\,(\text{temperature}) - 0.020\,(\text{kGy})(\text{temperature}) - 0.426\,(\text{kGy})^2 + 0.002\,(\text{temperature})^2$$

$$\text{R-Square} = 0.975$$

This equation predicts that the reduction in the number of surviving CFU of *S. aureus* ATCC 13565 (log (N/N_o)) at an absorbed dose of 1.50 kGy would be −2.02, −2.94, −3.45, −3.56, and −3.28 at irradiation temperatures of −20, −10, 0, +10, and +20°C, respectively. This equation also predicts that the reduction in surviving CFU at an absorbed dose of 3.0 kGy would be −6.30, −7.50, −8.31, −8.72, and −8.73 log CFU at irradiation temperatures of −20, −10, 0, +10, and +20°C, respectively.

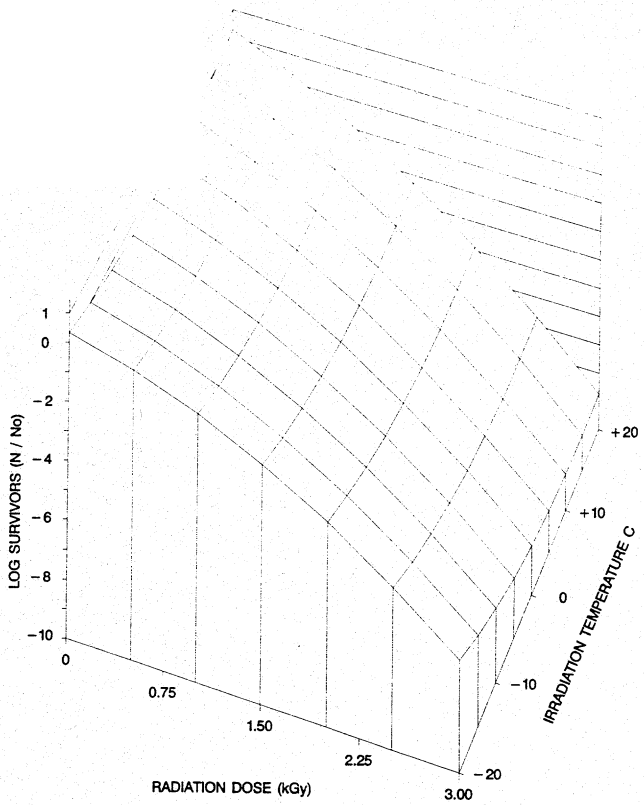

Fig. 3—Predicted survival of S. aureus *ATCC 13565 in mechanically deboned chicken meat to gamma radiation doses applied at −20°C to +20°C.*

Table 2.—Challenge study with S. aureus *ATCC 13565 in mechanically deboned chicken meat*

Hours abuse at 35°C		Radiation dose (kGy)				
		0	0.75	1.50	2.25	3.0
0	CFU[a]	3.86	0.79	ND[b]	ND	ND
	enterotoxin	neg	neg	neg	neg	neg
20	cfu	7.48	5.78	ND	ND	ND
	enterotoxin	pos	neg	neg	neg	neg

[a] log_{10}CFU/g of mechanically deboned chicken meat
[b] ND = not determined; neg = negative; pos = positive

Challenge study

Viable cells were found after exposure of MDCM inoculated with $10^{3.9}$ cells/g of *S. aureus* ATCC 13565 to 0.75 kGy but not after 1.50 kGy exposure. The results were not altered except by an increase in the number of cfu by abusive storage of the samples for 20 hr at 35°C. Enterotoxin was found only in the non-irradiated samples that were subjected to abuse for 20 hr at 35°C (Table 2).

DISCUSSION

ERDMAN et al. (1961) reported that the strains of *S. aureus* they examined had greater resistance to gamma radiation than did salmonellae. This appeared questionable as such results were not compatible with the results we obtained with *S. aureus* ATCC 13565. Further, Erdman et al. (1961) and Tiwari and Maxcy (1972) reported D-values for *S. aureus* in broth that were significantly higher than those we obtained in MDCM. The resistance (D-value = 0.36 kGy) of stationary phase *S.*

aureus ATCC 13565 to gamma radiation when mixed with MDCM was lower than most serovars of *Salmonella* reported by Thayer et al. (1990). They reported that the D-values for 6 serovars of *Salmonella* mixed with MDCM ranged from 0.37 to 0.77 kGy, (average 0.56 kGy). They also reported that the D-values for 6 serovars of *Salmonella* were significantly lower when suspended in broth than when mixed with MDCM. We developed a hypothesis that *S. aureus* ATCC 13565 may be unusually sensitive to gamma radiation. To test this hypothesis we elected to use 4 strains of *S. aureus*, including another sampling of the 196E strain, all of which had been maintained in the USDA, Eastern Regional Research Center culture collection. The results (Fig. 2) indicate that the mixture of the 4 strains of *S. aureus* suspended in 0.1% peptone water were much more sensitive to gamma radiation than when mixed with MDCM. The D-values for the mixed cfu suspension in peptone were less than those reported by Thayer et al. (1990) for *Salmonella* serovars suspended in broth. Further, though the D-value calculated for the 4-strain mixture of *Staphylococcus* with MDCM was greater than that obtained for *S. aureus* ATCC 13565, it was well within the range of D-values Thayer et al. (1990) had reported for 6 serovars of *Salmonella* in MDCM and lower than the mean for those 6 *Salmonella* serovars. Actively multiplying CFU (log-phase cells) of *S. aureus* ATCC 13565 were much more sensitive to gamma radiation than were stationary-phase cells as had been reported for several other bacteria. We conclude that the resistance of *S. aureus* ATCC 13565 to gamma radiation was probably not atypical of other strains of foodborne *S. aureus.*

The results indicate that *S. aureus* ATCC 13565 was not markedly sensitive to the temperature of irradiation when mixed with MDCM. The temperature at which the product was irradiated (even within the range of -20 to $+20$°C) nevertheless significantly affects the destruction of *S. aureus* in MDCM. These effects can be predicted with high reliability by use of an equation developed from the data. These predictions were confirmed by a challenge test with abusive storage of the product at 35°C.

Clearly, from the results with the mixture of 4 strains of *S. aureus* some strains may be more resistant to gamma radiation than the chosen experimental strain of 196E *S. aureus* ATCC 13565. That mixture of strains gave a D-value of 0.47 kGy *in vacuo* at 0°C. From the estimated D-value, 0.47 *in vacuo* at 0°C, for the mixture of the 4 strains of *S. aureus*, we estimate that doses of 3.0 kGy and 1.5 kGy should destroy 6.3 and 3.2 logs of CFU/g, respectively. These doses correspond to the maximum and minimum recommended doses for irradiation of poultry in the U.S. Even the lower value would require an extraordinary contamination of the product and irradiation of contaminated meat should provide significant protection against *S. aureus*. This conclusion was reinforced by the results we obtained in the challenge study in which viable CFU from an initial inoculum of 10^4 CFU/g were found after a dose of 0.75 kGy but not after a dose of 1.5 kGy. The fact that enterotoxin was not found in any of the irradiated samples after temperature abuse should not be interpreted as indicating destruction of the enterotoxin by the radiation. Rather, it indicates that too few viable CFU remained to reproduce significant numbers of cfu and subsequently form enterotoxin in the meat. Rose et al. (1988) reported that 27–34% of staphylococcal enterotoxin A remained in minced beef slurries after a radiation dose of 8.0 kGy. Thus, a maximum radiation dose of 3.0 kGy should provide significant protection for the consumer against foodborne *S. aureus*, which in MDCM is very sensitive to gamma radiation and should be destroyed by the dose range currently approved for poultry irradiation.

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